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(57) Abstract

Nucleic acid sequences that are useful for detecting *Chlamydia pneumoniae* are herein provided. These sequences can be used in hybridization assays or amplification based assays designed to detect the presence of *Chlamydia pneumoniae* in a test sample. Additionally, the sequences can be provided as part of a kit.

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NUCLEIC ACID PRIMERS AND PROBES FOR DETECTING *Chlamydia pneumoniae*

Field of the Invention

The present invention relates to *Chlamydia pneumoniae* and, in particular, it relates to oligonucleotides for detecting *Chlamydia pneumoniae* in a test sample.

Background of the Invention

Three species within the genus *Chlamydia* are clinically important because of their ability to infect and cause disease in a human host. *Chlamydia trachomatis* has been reported as the most common sexually transmitted disease in industrial societies and causes genital infections in both men and women. *Chlamydia psittaci* is responsible for a variety of respiratory tract infections. The most recently characterized and clinically important member of the *Chlamydia* genus is *Chlamydia pneumoniae* (*C. pneumoniae*) which also is responsible for respiratory tract infections and has been associated with coronary artery disease.

Perhaps because of its fairly recent characterization, the predominant methods for detecting *C. pneumoniae* in a test sample include isolation of the organism in culture, and serology testing. Isolation may include growing the organism in tissue culture cells to produce inclusion bodies which are then detected by fluorescently staining the inclusion bodies using a labeled species-specific-antibody. Serological testing requires two samples from an individual suspected of being infected with *C. pneumoniae*. Two samples are necessary because a significant number of individuals have antibodies to *C. pneumoniae* and a rise in antibody titer to *C. pneumoniae* or a change in antibody class (e.g. IgM to IgG) is measured as an indication of a recent *C. pneumoniae* infection. Because a rise in antibody titer or a change in antibody class is measured, acute and convalescent serum samples are taken. Unfortunately, these samples are often times taken weeks or even months apart. Hence, detecting a *C. pneumoniae* infection can be a time consuming process. Accordingly, there is a need for methods and

reagents capable of detecting *C. pneumoniae* in a specific and timely manner.

Summary of the Invention

The present invention provides nucleic acid sequences that can be used to specifically detect *C. pneumoniae* by using these sequences as oligonucleotide probes and/or primers. Such primers or probes are designated SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13 and SEQ ID NO 14. Those skilled in the art will recognize that homologs of these sequences and combinations of these sequences can also be employed to detect *C. pneumoniae* in a test sample. Preferably, the sequences are employed in amplification reactions and can be provided in kits along with other reagents for performing an amplification reaction.

Methods provided by the present invention include hybridization assays as well as amplification based assays. Thus, according to one method, a method of detecting the presence of *C. pneumoniae* in a test sample may comprise the steps of (a) contacting the test sample with one or more of the sequences listed above, or their homologs; and (b) detecting hybridization between the above sequences and a *C. pneumoniae* target sequence as an indication of the presence of *C. pneumoniae* in the test sample.

According to another embodiment, a method for detecting the presence of *C. pneumoniae* in a test sample may comprise the steps of (a) forming a reaction mixture comprising nucleic acid amplification reagents, a test sample containing a *C. pneumoniae* target sequence, and at least one primer and one probe oligonucleotide selected from the group consisting of SEQ ID NOs. 2 and 5; SEQ ID NOs. 3 and 4; SEQ ID NOs. 2, 3 and 4; SEQ ID NOs. 2, 3 and 5; SEQ ID NOs. 2, 3, 4 and 5; SEQ ID NOs. 9 and 11; SEQ ID NOs. 10 and 12; SEQ ID NOs. 9, 10 and 11; SEQ ID NOs. 9, 10 and 12; or SEQ ID NOs. 9, 10, 11 and 12; (b) subjecting the mixture to hybridization conditions to generate at least one nucleic acid sequence complementary to the target sequence; (c) hybridizing the probe to the nucleic acid sequence complementary to the target sequence, so as to form a complex comprising the probe and the complementary nucleic

acid sequence; and (d) detecting the so-formed complex as an indication of the presence of *C. pneumoniae* in the sample.

According to another embodiment, the invention provides kits which comprise a set of oligonucleotide primers and probes, selected from the SEQ ID NOs. listed above, and amplification reagents.

Detailed Description of the Invention

As previously mentioned, the present invention provides nucleic acid sequences, methods for using these sequences and kits containing these sequences, all of which can be employed to specifically detect *C. pneumoniae.* The sequences provided are designated herein as SEQ ID NOs. 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14 and homologs thereof. These sequences are derived from a *C. pneumoniae* gene encoding a cysteine rich outer-membrane-protein (OMP) disclosed in Watson, M. W., *et. al.*, *Journal of Clinical Microbiology*, 29(6) p. 1188-1193 (1991) and a *C. pneumoniae* gene encoding a 76 Kilodalton protein (76 kD protein) disclosed in Perez-Melgosa, M., *et. al.*, *Infection and Immunity*, 62(3) p. 880-886 (1994).

With respect to the sequences herein provided, the term "homologs" means those sequences sharing about 80% homology with SEQ ID NOs. 2-7 and 9-14, and more preferably those sequences that share about 90% homology with SEQ ID NOs. 2-7 and 9-14. Thus, sequences that contain about 80% homology with the sequences provided herein and specifically hybridize with *C. pneumoniae* are intended to be within the scope of the present invention. For example, extensions of the present sequences, sequences that are shorter than the present sequences but contain a subset of the present sequences, and those sequences that deviate from the present sequences by minor base substitutions are contemplated as within the scope of the present invention.

Those skilled in the art will recognize various modifications that can be made to the sequences designated SEQ ID NOs. 2-7 and 9-14 without departing from their ability to specifically detect *C. pneumoniae* and share about 80% homology with these sequences. For example, 3' or 5' extensions of the present sequences with bases that are complementary to succeeding or preceding bases in either the OMP gene or 76 kD protein gene are considered to be homologs of the present

sequences when they share about 80% homology with the present sequences and specifically detect *C. pneumoniae*. Additionally, 3' or 5' extensions of present sequences with bases that are not complementary to succeeding or preceding bases in the OMP gene or 76 kD protein gene that share about 80% homology with the present sequences and specifically detect *C. pneumoniae* are contemplated as within the scope of the present invention. Further, base substitutions can be made to SEQ ID NOs. 2-7 and 9-14, but these modified sequences will nevertheless maintain the ability to specifically hybridize with *C. pneumoniae* and share about 80% homology with SEQ ID NOs. 2-7 and 9-14. They are therefore contemplated as within the scope of the present invention. Moreover, sequences that contain about 80% of the sequences designated SEQ ID NOs. 2-7 and 9-14 but have bases deleted from the 3' or 5' end, are considered to be within the scope of the term homolog.

The sequences disclosed herein, as well as their homologs, may comprise deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or nucleic acid analogs such as uncharged nucleic acid analogs including but not limited to peptide nucleic acids (PNAs) which are disclosed in International Patent Application WO 92/20702 or morpholino analogs which are described in U.S. Patents Numbered 5,185,444, 5,034,506, and 5,142,047 all of which are herein incorporated by reference. Such sequences can routinely be synthesized using a variety of techniques currently available. For example, a sequence of DNA can be synthesized using conventional nucleotide phosphoramidite chemistry and the instruments available from Applied Biosystems, Inc. (Foster City, CA); DuPont, (Wilmington, DE); or Milligen, (Bedford, MA). Similarly, and when desirable, the sequences can be labeled using methodologies well known in the art such as described in U.S. Patent Applications Numbered 5,464,746; 5,424,414; and 4,948,882 all of which are herein incorporated by reference.

The term "label" as used herein means a molecule or moiety having a property or characteristic which is capable of detection. A label can be directly detectable, as with, for example, radioisotopes, fluorophores, chemiluminophores, enzymes, colloidal particles, fluorescent microparticles and the like; or a label may be indirectly detectable, as with, for example, specific binding members. It will be understood that directly detectable labels may require additional

components such as, for example, substrates, triggering reagents, light, and the like to enable detection of the label. When indirectly detectable labels are used, they are typically used in combination with a "conjugate". A conjugate is typically a specific binding member which has been attached or coupled to a directly detectable label. Coupling chemistries for synthesizing a conjugate are well known in the art and can include, for example, any chemical means and/or physical means that does not destroy the specific binding property of the specific binding member or the detectable property of the label. As used herein, "specific binding member" means a member of a binding pair, i.e., two different molecules where one of the molecules through, for example, chemical or physical means specifically binds to the other molecule. In addition to antigen and antibody specific binding pairs, other specific binding pairs include, but are not intended to be limited to, avidin and biotin; haptens and antibodies specific for haptens; complementary nucleotide sequences; enzyme cofactors or substrates and enzymes; and the like.

Generally, the sequences provided herein can be employed to detect the presence of *C. pneumoniae* in a test sample by contacting a test sample with at least one of the sequences provided herein under hybridizing conditions and detecting hybridization between the *C. pneumoniae* target sequence and at least one of the sequences designated herein as SEQ ID NOs. 2-7 and 9-14. Several well known methods for detecting hybridization can be employed according to the present invention and may include, for example, the use of gels and stains or detecting a label associated with one or more of the sequences provided herein after performing, for example, a dot blot or amplification reaction.

The term "test sample" as used herein, means anything suspected of containing the target sequence. The test sample can be derived from any biological source, such as for example, blood, bronchial alveolar lavage, saliva, throat swabs, ocular lens fluid, cerebral spinal fluid, sweat, sputa, urine, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, amniotic fluid, tissues such as heart tissue and the like, or fermentation broths, cell cultures, chemical reaction mixtures and the like. The test sample can be used (i) directly as obtained from the source or (ii) following a pre-treatment to modify the character of

the sample. Thus, the test sample can be pre-treated prior to use by, for example, preparing plasma from blood, disrupting cells, preparing liquids from solid materials, diluting viscous fluids, filtering liquids, distilling liquids, concentrating liquids, inactivating interfering components, adding reagents, and the like.

A "target sequence" as used herein means a nucleic acid sequence that is detected, amplified, both amplified and detected or otherwise is complementary to one of the sequences herein provided. Thus, a target sequence will be approximately 80% complementary with the sequences provided herein. Additionally, while the term target sequence is sometimes referred to as single stranded, those skilled in the art will recognize that the target sequence may actually be double stranded.

"Hybridization" or "hybridizing" conditions are defined generally as conditions which promote annealing between complementary nucleic acid sequences or annealing and extension of one or more nucleic acid sequences. It is well known in the art that such annealing is dependent in a rather predictable manner on several parameters, including temperature, ionic strength, sequence length and G:C content of the sequences. For example, lowering the temperature in the environment of complementary nucleic acid sequences promotes annealing. For any given set of sequences, melt temperature, or Tm, can be estimated by any of several known methods. Typically, diagnostic applications utilize hybridization temperatures which are slightly below the melt temperature. Ionic strength or "salt" concentration also impacts the melt temperature, since small cations tend to stabilize the formation of duplexes by negating the negative charge on the phosphodiester backbone. Typical salt concentrations depend on the nature and valency of the cation but are readily understood by those skilled in the art. Similarly, high G:C content and increased sequence length are also known to stabilize duplex formation because G:C pairings involve 3 hydrogen bonds where A:T pairs have just two, and because longer sequences have more hydrogen bonds holding the sequences together. Thus, a high G:C content and longer sequence lengths impact the hybridization conditions by elevating the melt temperature.

Once sequences are selected for a given diagnostic application, the G:C content and length will be known and can be accounted for in determining precisely what hybridization conditions will encompass.

Since ionic strength is typically optimized for enzymatic activity, the only parameter left to vary is the temperature. For improved specificity, the hybridization temperature is selected slightly below the Tm of the primers or probe; typically 2-10°C below the Tm. Thus, obtaining suitable hybridization conditions for a particular primer, probe or primer and probe set is well within ordinary skill of one practicing this art.

The sequences provided herein also can be used as amplification primers or probes according to amplification procedures well known in the art. Such reactions include, but are not intended to be limited to, the polymerase chain reaction (PCR) described in U.S. Patents 4,683,195 and 4,683,202, the ligase chain reaction (LCR) described in EP-A-320 308, and gap LCR (GLCR) described in U.S. Patent No. 5,427,930 all of which are herein incorporated by reference.

According to a preferred embodiment, the sequences are employed in the "oligonucleotide hybridization PCR" (variably referred to herein as "OH PCR") amplification reaction as described in U.S. Patent Application Serial No. 08/514,704, filed August 14, 1995, that is herein incorporated by reference. Briefly, the reagents employed in the preferred method comprise at least one amplification primer and at least one internal hybridization probe, as well as other reagents for performing an amplification reaction.

The primer sequence is employed to prime extension of a copy of a target sequence and is labeled with either a capture label or a detection label. The probe sequence is used to hybridize with the sequence generated by the primer sequence, and typically hybridizes with a sequence that does not include the primer sequence. Similarly to the primer sequence, the probe sequence is also labeled with either a capture label or a detection label with the caveat that when the primer is labeled with a capture label the probe is labeled with a detection label and vice versa. Detection labels have the same definition as "labels" previously defined and "capture labels" are typically used to separate extension products, and probes associated with any such products, from other amplification reactants. Specific binding members (as previously defined) are well suited for this purpose. Also, probes used according to this method are preferably blocked at their 3' ends so that they are not extended under hybridization conditions.

Methods for preventing extension of a probe are well known and are a matter of choice for one skilled in the art. Typically, adding a phosphate group to the 3' end of the probe will suffice for purposes of blocking extension of the probe.

"Other reagents for performing an amplification reaction" or "nucleic acid amplification reagents" include reagents which are well known and may include, but are not limited to, an enzyme having polymerase activity, enzyme cofactors such as magnesium; salts; nicotinamide adenine dinucleotide (NAD); and deoxynucleotide triphosphates (dNTPs) such as for example deoxyadenine triphosphate, deoxyguanine triphosphate, deoxycytosine triphosphate and deoxythymine triphosphate.

The preferred method generally comprises the steps of (a) forming a reaction mixture comprising nucleic acid amplification reagents, at least one hybridization probe, at least one amplification primer and a test sample suspected of containing a target sequence; (b) subjecting the mixture to hybridization conditions to generate at least one copy of a nucleic acid sequence complementary to the target sequence; (c) hybridizing the probe to the nucleic acid sequence complementary to the target sequence, so as to form a hybrid comprising the probe and the nucleic acid sequence complementary to the target sequence; and (d) detecting the hybrid as an indication of the presence of *C. pneumoniae* in the sample. It will be understood that step (b) of the above method can be repeated several times prior to step (c) by thermal cycling the reaction mixture as is well known in the art.

According to the above method, it is preferable to select primers and probes such that the probe sequence has a lower melt temperature than the primer sequences so that upon placing the reaction mixture under hybridization conditions copies of the target sequence or its complement are produced at temperature above the Tm of the probe. After such copies are synthesized, they are denatured and the mixture is cooled to enable the formation of hybrids between the probes and single stranded copies of the target or its complement. The rate of temperature reduction from the denaturation temperature down to a temperature at which the probes will bind to single stranded copies is preferably quite rapid (for example 8 to 15 minutes) and particularly through the temperature range in which an enzyme having polymerase

TABLE 1

Sample	LCx® rate (c/s/s)
C. psittaci	26.2
C. trachomatis	23.9
C. pneumoniae (Positive Control)	1144.1

B. Specific Detection of *C. pneumoniae* using the 76kD Primers and Probe The 76kD primers (SEQ ID NO 9 and SEQ ID NO 10) and 76kD detection probe (SEQ ID NO 11) described in Example 1 were used to amplify and detect 3 samples from the genus *Chlamydia* (TABLE 2) by the method described in 2.B. above. The data from this experiment is presented in TABLE 2 and shows specific amplification and detection of *C. pneumoniae* only, with the 2 other *Chlamydia* genus samples being non-reactive.

TABLE 2

Sample	LCx® rate (c/s/s)
C. psittaci	47.1
C. trachomatis	34.5
C. pneumoniae (Positive Control)	994.0

Example 4

Sensitivity of *C. pneumoniae* Detection

A panel of *C. pneumoniae* cells which had been quantified using immunofluorescence to determine the number of Inclusion Forming Units (IFU) in each sample, were lysed and tested by the current methodology. Salmon sperm DNA was used as a negative control and the *C. pneumoniae* standard DNA as a positive control.

A. Sensitivity of the *C. pneumoniae* OMP Primers and Probe The OMP primers (SEQ ID NO 2 and SEQ ID NO 3) and OMP detection probe (SEQ ID NO 4), described in Example 1, were used to amplify and detect a quantified panel of *C. pneumoniae* cells (TABLE 3) by a unit dose modification of the method used in Examples 2 and 3, namely: the primers, at a concentration of 0.3 µM each, detection probe, at a

concentration of 8 nM, as well as the other reagents were added to a single amplification vessel. Taq polymerase was used at a concentration of 2.5 units. PCR extension was performed in 10X PCR buffer (Perkin Elmer, Foster City, CA) which consists of 100 mM Tris-HCl, pH 8.3, 500 mM KCl, at a final concentration of 1X. The final concentration of $MgCl_2$ was 2 mM and the final concentration of the nucleotides was 0.2 mM each, in a total reaction volume of 0.2 ml.

The reaction mixture was amplified in a Perkin-Elmer 480 Thermal Cycler under the following cycling conditions: 97°C for 30 seconds/59°C for 30 seconds/72°C for 30 seconds for 40 cycles. After maintaining the reaction mixture at 97°C for 5 minutes, probe oligo hybridization was accomplished by lowering the temperature to 15°C for 10 minutes.

Following probe hybridization, reaction products were detected on the Abbott LCx® system. The data from this experiment is presented in TABLE 3 and shows detection of *C. pneumoniae* at concentrations as low as 0.06 IFU/reaction.

TABLE 3

Sample #	*C. pneumoniae* (IFU/reaction)	LCx® rate (c/s/s)
1	50000.00	2305
2	12500.00	2320
3	15625.00	2341
4	3906.25	2215
5	976.56	2361
6	244.14	2262
7	61.04	2329
8	15.26	2262
9	3.81	2302
10	0.95	2241
11	0.06	1804
12	0.05	29

Additional testing was performed in triplicate at concentrations below 1 IFU/reaction. The results, shown in TABLE 4, indicate consistent detection of *C. pneumoniae* at concentrations of 0.38 IFU/reaction.

TABLE 4

Sample #	*C. pneumoniae* (IFU/reaction)	LCx® rate (c/s/s)
1	0.38	2477
1	0.38	2277
1	0.38	2414
2	0.10	33
2	0.10	1764
2	0.10	2414
3	0.02	34
3	0.02	31
3	0.02	29
Negative Control		77
Negative Control		70
Negative Control		89
Positive Control		1876
Positive Control		1987
Positive Control		1919

B. Sensitivity of the *C. pneumoniae* 76kD Primers and Probe The 76kD primers (SEQ ID NO 9 and SEQ ID NO 10) and 76kD detection probe (SEQ ID NO 11), described in Example 1, were used to amplify and detect a quantified panel of *C. pneumoniae* cells (TABLE 5) by the unit dose method described in Example 4.A. above. The primers were used at a concentration of 0.3 μM and the detection probe was used at a concentration of 8 nM. The other reaction mixture components were the same as in 4.A. above with the exception of $MgCl_2$ which was used at a final concentration of 1 mM.

The reaction mixture was amplified, followed by probe oligo hybridization as in 4.A. above.

Following probe hybridization, reaction products were detected on the Abbott LCx® system. The data from this experiment is presented in TABLE 5 and shows detection of *C. pneumoniae* at concentrations as low as 0.05 IFU/reaction.

TABLE 5

Sample #	*C. pneumoniae* (IFU/reaction)	LCx® rate (c/s/s)
1	50000.00	1657
2	12500.00	1776
3	15625.00	1686
4	3906.25	1624
5	976.56	1685
6	244.14	1646
7	61.04	4688
8	15.26	1622
9	3.81	1628
10	0.95	1522
11	0.06	21
12	0.05	984
Negative Control		41
Positive Control		576

Additional testing was performed in triplicate at concentrations below 1 IFU/reaction. The results shown in TABLE 6 indicate consistent detection of *C. pneumoniae* at concentrations of 0.38 IFU/reaction.

TABLE 6

Sample #	*C. pneumoniae* (IFU)	LCx® rate (c/s/s)
1	0.38	1488
1	0.38	1410
1	0.38	1378
2	0.10	26
2	0.10	25
2	0.10	560
3	0.02	27
3	0.02	21
3	0.02	31
Negative Control		26
Negative Control		30
Negative Control		34
Positive Control		1531
Positive Control		1572
Positive Control		47

Example 5

Sensitivity and Specificity of *C. pneumoniae* OMP and 76kD Primers and Probes

The OMP primers (SEQ ID NO 2 and SEQ ID NO 3) and OMP detection probe (SEQ ID NO 4) or the 76kD primers (SEQ ID NO 9 and SEQ ID NO 10) and 76kD detection probe (SEQ ID NO 11), as described in Example 1, were used to amplify and detect previously quantified genomic DNA from both *C. pneumoniae* and *Mycoplasma pneumoniae* (*M. pneumoniae*), using the respective methods in Example 4 above. The data from this experiment is presented in TABLE 7 and shows detection of *C. pneumoniae* by both OMP and 76kD primer/probe sets at genomic DNA concentrations of 15.6 pg/ml, with no cross-detection of *M. pneumoniae* genomic DNA.

TABLE 7

Sample	Genomic DNA (pg/ml)	OMP LCx® rate (c/s/s)	76kD LCx® rate (c/s/s)
C. pneumoniae	5000	2417	1864
	1250	2438	1882
	312	2543	1827
	78	2420	1772
	15.6	2481	1653
M. pneumoniae	5000	37	20
	1250	38	22
	312	46	18
	78	34	26
	15.6	41	30
Buffer	0	38	21

Example 6

Comparison of *C. pneumoniae* Detection by OH-PCR and Culture

A. OH-PCR and Culture Detection of *C. pneumoniae* in nasopharyngeal swab samples. Test results from twenty-five nasopharyngeal swab samples obtained from patients that were tested for *C. pneumoniae* by traditional culture methodology were compared to results obtained using OMP primers (SEQ ID NO 2 and SEQ ID NO 3) and OMP detection probe (SEQ ID NO 4) or the 76kD primers (SEQ ID NO 9 and SEQ ID NO 10) and 76kD detection probe (SEQ ID NO 11) as described in Example 1. Sample DNA was isolated using the QIAgen nucleic acid purification method and amplified and detected by the respective OMP or 76kD methods as in Example 4 above. Results are shown in Table 8. *C. pneumoniae* was used as a positive control and salmon sperm DNA was used as a negative control.

TABLE 8

Sample #	Culture	OMP LCx® rate (c/s/s)	76kD LCx® rate (c/s/s)
1	-	23	32
2	-	952	644
3	-	18	24
4	-	37	24
5	-	20	26
6	-	1499	2180
7	-	23	25
8	-	24	19
9	-	23	24
10	-	23	25
11	-	29	22
12	+	1538	2188
13	-	14	24
14	-	25	25
15	+	1510	2264
16	+	1670	2190
17	+	1532	2140
18	+	1455	2107
19	-	22	28
20	+	1609	2258
21	+	1580	2237
22	+	1525	2226
23	-	19	20
24	+	2348	1393
25	+	2215	1464
Neg Control		24	85
Neg Control		78	30
Pos Control		1568	2061
Pos Control		2048	1353

activity is active for primer extension. Such a rapid cooling copy sequence/probe hybridization rather that primer/copy seq. hybridization.

Upon formation of the copy sequence/probe hybrids, the differential labels (i.e. capture and detection labels) on the copy sequence and probe sequence can be used to separate and detect such hybrids. Preferably, detection is performed according to the protocols used by the commercially available Abbott LCx® instrumentation (Abbott Laboratories; Abbott Park, IL).

Thus, keeping the preferred method in mind, the sequences of the present invention are preferably provided in groups of at least two different sequences (i.e. at least one primer sequence and at least one probe sequence complementary to the extension product of the primer). Hence, SEQ ID NOs. 2 and 5; SEQ ID NOs. 3 and 4; SEQ ID NOs. 2, 3 and 4; SEQ ID NOs. 2, 3 and 5; SEQ ID NOs. 2, 3, 4 and 5; SEQ ID NOs. 9 and 11; SEQ ID NOs. 10 and 12; SEQ ID NOs. 9, 10 and 11; SEQ ID NOs. 9, 10 and 12; or SEQ ID NOs. 9, 10, 11 and 12; or homologs of these sequences are preferably provided together.

The sequences of the present invention can be provided as part of a kit useful for detecting *C. pneumoniae* The kits comprise one or more suitable containers containing one or more sequences according to the present invention, an enzyme having polymerase activity, and deoxynucleotide triphosphates. Typically, at least one sequence bears a label, but detection is possible without this.

The following examples are provided to further illustrate the present invention and not intended to limit the invention.

Examples

The following examples demonstrate use of the DNA oligomer primers and probes provided herein for detecting *C. pneumoniae*. The primers and probes used in the examples are identified as SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 9, SEQUENCE ID NO 10, and SEQUENCE ID NO 11. SEQUENCE ID NOs 2, 3 and 4 are specific for the gene encoding the 60kD cysteine rich outer major protein (OMP) of *C. pneumoniae*, a portion of which is designated herein as SEQ ID NO 1. SEQUENCE ID NO 9, 10 and 11 are specific for the gene encoding the 76kD protein of *C. pneumoniae*, a portion of which is

designated herein as SEQ ID NO 8. In the following examples, SEQUENCE ID NOs 2 and 3 are used as *C. pneumoniae* amplification primers specific for the OMP region. SEQ ID NO 4 is used as an internal hybridization probe for the OMP amplification product. SEQ ID NOs 9 and 10 are used as amplification primers specific for the 76kD region of *C. pneumoniae* and SEQ ID NO 11 is used as an internal hybridization probe for the 76kD amplification product.

In the following examples, "positive-control *C. pneumonia* sequences" (variably referred to as the "*C. pneumoniae* standard") were derived from *C. pneumoniae* cell lines TW-183, AR-39 and CWL-029 (obtained from the American Type Culture Collection -ATCC-, Rockville, MD). The sequences were obtained by mixing equal numbers of cells from all three cell lines and collecting DNA with the QIAgen nucleic acid purification method (QIAgen, Inc., Chatsworth, CA).

Example 1

Preparation of *C. pneumoniae* Primers and Probes

A. OMP Primers and Probe Target-specific primers and probes were designed to detect the *C. pneumoniae* OMP target sequence by oligonucleotide hybridization PCR. The primers were SEQUENCE ID NO 2 and SEQUENCE ID NO 3. Primer sequences were synthesized using standard oligonucleotide synthesis methodology and haptenated with adamantane at their 5' ends using standard cyanoethyl phosphoramidite coupling chemistry as described in U.S. Patent No. 5,424,414 incorporated herein by reference.

The detection probe was designed to hybridize with the amplified *C. pneumoniae* OMP target sequence by oligonucleotide hybridization. This probe is SEQUENCE ID NO 4. The probe sequence was synthesized using standard oligonucleotide synthesis methodology and haptenated with 2 carbazoles at the 5' end using standard cyanoethyl phosphoramidite coupling chemistry as described in U.S. Patent No. 5,464,746 (herein incorporated by reference), and blocked with phosphate at the 3' end. Reactivity was assessed against the *C. pneumoniae* standard.

B. 76kD Primers and Probe Target-specific primers and probes were designed to detect the *C. pneumoniae* 76kD target sequence by

oligonucleotide hybridization PCR. The primers were SEQUENCE ID NO 9 and SEQUENCE ID NO 10. Primer sequences were synthesized using standard oligonucleotide synthesis methodology and haptenated with adamantane at their 5' ends using standard cyanoethyl phosphoramidite coupling chemistry U.S. Patent No. 5,424,414.

The detection probe was designed to hybridize with the amplified *C. pneumoniae* 76kD target sequence by oligonucleotide hybridization. This probe is SEQUENCE ID NO 11. The probe sequence was synthesized using standard oligonucleotide synthesis methodology and haptenated with 2 carbazoles at the 5' end using standard cyanoethyl phosphoramidite coupling chemistry (as above) and blocked with phosphate at the 3' end. Reactivity was assessed against the *C. pneumoniae* standard.

Example 2

Amplification and Detection of *C. pneumoniae*

A. *C. pneumoniae* OMP Detection. The *C. pneumoniae* standard sample was PCR amplified and detected using the OMP primers (SEQ ID NO 2 and 3) and OMP detection probe (SEQ ID NO 4) described in Example 1.A. The primers were used at a concentrations of 0.2 µM each. Taq polymerase was used at a concentration of 2.5 units. PCR extension was performed using 10X PCR buffer (Perkin Elmer, Foster City, CA) which consists of 100 mM Tris-HCl, pH 8.3, 500 mM KCl, at a final concentration of 1X. The final concentration of $MgCl_2$ was 2 mM and the final concentration of the nucleotides was 0.2 mM each, in a total reaction volume of 0.2 ml.

The reaction mixture was amplified in a Perkin-Elmer 480 Thermal Cycler under the following cycling conditions: 97°C for 30 seconds/59°C for 30 seconds/72°C for 30 seconds for 40 cycles.

Following amplification, a 100 µl aliquot from the above reaction mixture was added to a separate tube containing 10 µl of the detection probe at a concentration of 40 nM (therefore final detection probe concentration was 3.6 nM). After an initial denaturation step at 97°C for 5 minutes, probe oligo hybridization was accomplished by lowering the temperature to 15°C for 10 minutes.

Following probe hybridization, reaction products were detected on the Abbott LCx® system (available from Abbott Laboratories, Abbott

Park, IL). A suspension of anti-carbazole antibody coated microparticles and an anti-adamantane antibody/alkaline phosphatase conjugate (all of which are commercially available from Abbott Laboratories, Abbott Park, IL) were used in conjunction with the LCx® to capture and detect the reaction products. The LCx® showed a positive reaction rate of 1144.1 c/s/s using the OMP primer/probe set to detect *C. pneumoniae*.

B. *C. pneumoniae* 76kD detection. The *C. pneumoniae* standard sample was PCR amplified and detected using the 76kD primers (SEQ ID NO 9 and 10) and 76kD detection probe (SEQ ID NO 11) described in Example 1.B. Concentrations of reagents used in this example were the same as those used in Example 2.A. above.

The reaction mixture was amplified, followed by probe oligo hybridization as in 2.A. above.

Following probe hybridization, reaction products were detected on the Abbott LCx® system, as above in Example 2.A. The LCx® showed a positive reaction rate of 994.0 c/s/s using the 76kD primer/probe set to detect *C. pneumoniae*.

Example 3

Specificity of *C. pneumoniae* Detection

DNA from two other members of the genus *Chlamydia*, *C. psittaci* and *C. trachomatis*, was purchased from ABI (Advanced Biotechnologies, Inc., Columbia, MD), diluted to levels representing 7.1×10^4 and 1.26×10^5 elementary bodies, respectively, and assayed side by side with the *C. pneumoniae* standard from Example 2, as described below.

A. Specific Detection of *C. pneumoniae* using the OMP Primers and Probe The OMP primers (SEQ ID NO 2 and SEQ ID NO 3) and OMP detection probe (SEQ ID NO 4) described in Example 1 were used to amplify and detect 3 samples from the genus *Chlamydia* (TABLE 1) by the method described in 2.A. above. The data from this experiment is presented in TABLE 1 and shows specific amplification and detection of *C. pneumoniae* only, with the 2 other *Chlamydia* genus samples being non-reactive.

Ten samples were identified as positive for C. pneumoniae by culture (#12, 15, 16, 17,18, 20, 21, 22, 24 and 25), all of which were also detected as positive by both OMP and 76kD assay methods. Two additional samples (#2 and 6) were found positive by both the OMP and 76kD *C. pneumoniae* primer/probe sets using OH-PCR on the LCx®.

B. Detection of *C. pneumoniae* in Throat Swab and Nasopharyngeal Swab Samples Using the OMP Primer/Probe Set and Culture Eighteen paired throat swab and nasopharyngeal swab samples obtained from patients were tested for *C. pneumoniae* by traditional culture methodology and compared to *C. pneumoniae* detection using OMP primers (SEQ ID NO 2 and SEQ ID NO 3) and OMP detection probe (SEQ ID NO 4) as described in Example 1. Sample DNA was isolated using the QIAgen nucleic acid purification method and amplified and detected by the OMP method as in Example 4.A. above.

The results using the OMP *C. pneumoniae* primer/probe set showed concordance with standard culture, with all samples negative by both methods.

While the invention has been described in detail and with reference to specific embodiments, it will be apparent to one skilled in the art that various changes and modifications may be made to such embodiments without departing from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: M. B. Cerney

(ii) TITLE OF INVENTION: NUCLEIC ACID SEQUENCES FOR DETECTING CHLAMYDIA PNEUMONIAE

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Abbott Laboratories
(B) STREET: 100 Abbott Park Road
(C) CITY: Abbott Park
(D) STATE: Illinois
(E) COUNTRY: USA
(F) ZIP: 60064-3500

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Macintosh
(C) OPERATING SYSTEM: System 7.0.1
(D) SOFTWARE: Microsoft Word 5.1a

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Paul D. Yasger
(B) REGISTRATION NUMBER: 37,477
(C) DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 708/937-2341
(B) TELEFAX: 708/938-2623
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 230 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (C. pneumoniae)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TAGAAATTTG CCAGTCCGTT CCAGAATACG CTACTGTAGG ATCTCCTTAC 50

CCTATTGAAA TCCTTGCTAT AGGCAAAAAA GATTGTGTTG ATGTTGTGAT 100

TACACAACAC CTACCTTGCG AAGCTGAATT CGTAAGCAGT GATCCAGAAA 150

CAACTCCTAC AAGTGATGGG AAATTAGTCT GGAAAATCGA TCGCCTGGGT 200

GCAGGAGATA AATGCAAAAT TACTGTATGG 230

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGTCCGTTC CAGAATACGC TACTG 25

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGCATTTATC TCCTGCACCC AGG 23

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCAGAAACAA CTCCTACAAG 20

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTTGTAGGAG TTGTTTCTGG 20

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGTAGCGTA TTCTGGAACG GACTG 25

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCTGGGTGCA GGAGATAAAT GCA 23

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA (C. pneumoniae)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TACCTCAACA TCACTAGCTG ACATACAGGC TGCTTTGGTG AGCCTCCAGG 50

ATGCTGTCAC TAATATAAAG GATACAGCGG CTACTGATGA GGAAACCGCA 100

ATCGCTGCGG TGTGGGAAAC TAAGAATGCC GATGCAGTTA AAGTTGGCGC 150

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTAGCTGACA TACAGGCTGC TTTGG 25

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATCGGCATT CTTAGTTTCC CACTC 25

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTCCTCATCA GTAGCC 16

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCTACTGAT GAGGAA 16

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCAAAGCAGC CTGTATGTCA GCTAG 25

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGTGGGAAA CTAAGAATGC CGATG 25

Claims

What is claimed is:

1. An oligonucleotide primer or probe, said primer or probe selected from the group consisting of: SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, homologs thereof, and combinations thereof.

2. The combination of oligonucleotides of claim 1 selected from the group consisting of: SEQ ID NOs. 2 and 5; SEQ ID NOs. 3 and 4; SEQ ID NOs. 2, 3 and 4; SEQ ID NOs. 2, 3 and 5; SEQ ID NOs. 2, 3, 4 and 5; SEQ ID NOs. 9 and 11; SEQ ID NOs. 10 and 12; SEQ ID NOs. 9, 10 and 11; SEQ ID NOs. 9, 10 and 12; or SEQ ID NOs. 9, 10, 11 and 12.

3. A method of detecting the presence of *C. pneumoniae* in a test sample comprising the steps of:

a) contacting said test sample with an oligonucleotide of claim 1; and

b) detecting hybridization between said oligonucleotide and a *C. pneumoniae* target sequence as an indication of the presence of *C. pneumoniae* in said test sample.

4. The method of claim 3 wherein said oligonucleotide is labeled.

5. A method for detecting the presence of *C. pneumoniae* in a test sample comprising the steps of:

a) forming a reaction mixture comprising nucleic acid amplification reagents, a test sample containing a *C. pneumoniae* target sequence, and at least one primer and one probe oligonucleotide selected from the group consisting of SEQ ID NOs. 2 and 5; SEQ ID NOs. 3 and 4; SEQ ID NOs. 2, 3 and 4; SEQ ID NOs. 2, 3 and 5; SEQ ID NOs. 2, 3, 4 and 5; SEQ ID NOs. 9 and 10; SEQ ID NOs. 10 and 12; SEQ ID NOs. 9, 10 and 11; SEQ ID NOs. 9, 10 and 12; SEQ ID NOs. 9, 10, 11 and 12; and homologs thereof; and

b) subjecting said mixture to hybridization conditions to generate at least one nucleic acid sequence complementary to said target sequence;

c) hybridizing said probe to said nucleic acid complementary to said target sequence, so as to form a hybrid comprising said probe and said nucleic acid; and

d) detecting said hybrid as an indication of the presence of *C. pneumoniae* in said sample.

6. The method of claim 5 wherein said probe is labeled.

7. The method of claim 5 wherein said probe is labeled with a capture label and said primer is labeled with a detection label.

8. The method of claim 5 wherein said probe is labeled with a detection label and said primer is labeled with a capture label.

9. A kit comprising:

a) an oligonucleotide selected from the group consisting of SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, homologs thereof, and combinations thereof, and

b) amplification reagents.

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(74) Agents: YASGER, Paul, D. et al.; Abbott Laboratories, CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

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(54) Title: NUCLEIC ACID PRIMERS AND PROBES FOR DETECTING *CHLAMYDIA PNEUMONIAE*

(57) Abstract

Nucleic acid sequences that are useful for detecting *Chlamydia pneumoniae* are herein provided. These sequences can be used in hybridization assays or amplification based assays designed to detect the presence of *Chlamydia pneumoniae* in a test sample. Additionally, the sequences can be provided as part of a kit.

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SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
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UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/US 97/09673

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 587 331 A (GEN PROBE INC) 16 March 1994 see the whole document ---	1-4
X	EP 0 577 144 A (WASHINGTON RES FOUND) 5 January 1994 see examples 8,9 ---	1-4
X	CAMPBELL L ET AL: "Detection of Chlamydia pneumoniae by Polymerase Chain Reaction" JOURNAL OF CLINICAL MICROBIOLOGY, vol. 30, no. 2, February 1992, pages 434-39, XP000406741 see the whole document --- -/--	1-9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 1998	29. 04. 98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Osborne, H

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 97/09673

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TJIHIE H ET AL: "Detection of Chlamydia pneumoniae using a general Chlamydia Polymerase Chain Reaction with species differentiation after hybridisation" JOURNAL OF MICROBIOLOGICAL METHODS, vol. 18, no. 2, September 1993, pages 137-150, XP000405961 see the whole document	1-9
X	GAYDOS C ET AL: "Diagnostic utility of PCR-enzyme immunoassay, culture, and serology for detection of Chlamydia pneumoniae" JOURNAL OF CLINICAL MICROBIOLOGY, vol. 32, no. 4, April 1994, pages 903-5, XP002047218 see page 904, paragraph 1	1-9
X	KHAN M ET AL: "A reverse transcriptase-PCR based assay for in-vitro susceptability testing of Chlamydia pneumoniae" JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol. 37, April 1996, pages 677-85, XP002047219 see the whole document	1-9
Y	PEREZ-MELGOSA M ET AL: "Isolation and characterization of a gene encoding a Chlamydia pneumoniae 76- kilodalton protein containing a species-specific epitope " INFECTION AND IMMUNITY, vol. 62, no. 3, March 1994, pages 880-86, XP002059939 cited in the application see the whole document	1-9
Y	WO 94 29486 A (SALK INST FOR BIOLOGICAL STUDI) 22 December 1994 see page 45, line 10 - page 50, line 5	1-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/09673

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/09673

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-9 searched partially in respect to SEQ ID NOS 2-7

   Oligonucleotide probes or primers derived from the nucleic acid sequence encoding the 60kD outer membrane protein of Chlamydia pneumoniae for the detection of said microorganism in hybridisation assays or an amplification based assays

2. Claims: 1-9, partially with respect to SEQ ID NOS 9-14.

   Oligonucleotide probes or primers derived from the nucleic acid sequence encoding the 76kD protein of Chlamydia pneumoniae for use in the detection of said microorganism in hybridisation assays or amplification based assays.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/US 97/09673

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0587331 A	16-03-94	US 5374718 A	20-12-94
		AU 5002993 A	15-03-94
		CA 2148468 A	03-03-94
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EP 0577144 A	05-01-94	AU 605485 B	17-01-91
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		DE 3789597 T	21-07-94
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		JP 1500083 T	19-01-89
		JP 2675564 B	12-11-97
		WO 8706617 A	05-11-87
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